- 2 -

residues, but this peptide proved incapable of sensitizing EBV-B cells to lysis by CTL 41. In contrast, peptides MEVDPIGHLY (SEQ ID NO:59) and EVDPIGHLY (SEQ ID NO:56) scored positive in this cytotoxicity assay and produced half-maximal lysis of autologous EBV-B target cells at ~0.05 nM (Fig. 11). This half-maximal lysis peptide concentration is lower than with the other MAGE antigenic peptides which produce half-maximal lysis at peptide concentrations from ~0.1 to ~25 nM (Chaux et al., *J. Immunol.* 163:2928, 1999; Traversari et al., *J. Exp. Med.* 176:1453, 1992; van der Bruggen et al., *Eur. J. Immunol.* 24:2134, 1994; Luiten and van der Bruggen, *Tissue Antigens* 55:149, 2000). Thus, the epitope recognized by CTL 41 does not contain consensus anchor residues for HLA-B35. It would therefore not have been discovered by an approach based on candidate peptides chosen on the basis of their sequence and used for *in vitro* stimulation of T lymphocytes. Peptides MEVDPIGHLY (SEQ ID NO:59) and EVDPIGHLY (SEQ ID NO:56) are encoded by the MAGE-A3 gene but not by another MAGE gene.

Remarks

During the preparation of a response to the Examiner's requirement for information under 37 C.F.R. 1.105, Applicants observed that the amino acid sequence of a MAGE-A3 peptide described in the application (SEQ ID NO:57), did not match the sequence of the portion of the MAGE-A3 protein from which it was derived. SEQ ID NO:57 was described as corresponding to MAGE-A3<sub>167-182</sub>. The sequence of the peptide was reported as MEVDPIGHLYIFACTL, while the correct sequence of this portion of the MAGE-A3 protein, as provided in SEQ ID NO:55 is: MEVDPIGHLYIFATCL. Applicants have amended the specification and submitted a revised Sequence Listing to correct this typographical error.

If the Examiner has any question concerning the foregoing amendment, the Examiner is invited to telephone the undersigned at the number listed below.

Respectfully submitted,

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600 Atlantic Avenue

Boston, Massachusetts 02210

Tel: (617) 720-3500

Attorney's Docket No. L00461/70104

Dated: August 9, 2002

**XNDD** 

## **Amended Paragraph**

Peptide [MEVDPIGHLYIFACTL] MEVDPIGHLYIFATCL (MAGE-A3167-182; SEQ ID NO:57) scored positive. The consensus anchor residues for HLA-B35 are P in position 2 and Y, F, M, L or I in position 9 (Rammensee, H.G., J. Bachmann, and S. Stevanovic. 1997. MHC Ligands and Peptide Motifs. Springer, New York). Peptide DPIGHLYIF (SEQ ID NO:58) contained the consensus anchor residues, but this peptide proved incapable of sensitizing EBV-B cells to lysis by CTL 41. In contrast, peptides MEVDPIGHLY (SEQ ID NO:59) and EVDPIGHLY (SEQ ID NO:56) scored positive in this cytotoxicity assay and produced halfmaximal lysis of autologous EBV-B target cells at  $\sim 0.05$  nM (Fig. 11). This half-maximal lysis peptide concentration is lower than with the other MAGE antigenic peptides which produce halfmaximal lysis at peptide concentrations from ~0.1 to ~25 nM (Chaux et al., J. Immunol. 163:2928, 1999; Traversari et al., J. Exp. Med. 176:1453, 1992; van der Bruggen et al., Eur. J. Immunol. 24:2134, 1994; Luiten and van der Bruggen, Tissue Antigens 55:149, 2000). Thus, the epitope recognized by CTL 41 does not contain consensus anchor residues for HLA-B35. It would therefore not have been discovered by an approach based on candidate peptides chosen on the basis of their sequence and used for in vitro stimulation of T lymphocytes. Peptides MEVDPIGHLY (SEQ ID NO:59) and EVDPIGHLY (SEQ ID NO:56) are encoded by the MAGE-A3 gene but not by another MAGE gene.

**SEQ** SEQ SEQ **SEQ** SEQ SEQ SEQ SEQ

ID NO:53 aligns with residues 161-169 of SEQ ID NO:2 ID NO:16 aligns with residues 162-169 of SEQ ID NO:2

ID NO:12 aligns with residues 148-169 of SEQ ID NO:2 ID NO:14 aligns with residues 161-169 of SEQ ID NO:2

ID NO:10 aligns with residues 162-169 of SEQ ID NO:2 ID NO:9 aligns with residues 160-169 of SEQ ID NO:2 ID NO:8 aligns with residues 161-169 of SEQ ID NO:2 ID NO:7 aligns with residues 163-171 of SEQ ID NO:2 ID NO:6 aligns with residues 163-172 of SEQ ID NO:2 ID NO:5 aligns with residues 161-172 of SEQ ID NO:2

## **TECH CENTER 1600/2900**

## Met Ser Glu Ser Leu Gln Leu Val Phe Gly Ile Asp Val Lys Glı Met Glı Met

Gly Lys Ala Ser Glu Ser Leu Gln Leu Val Phe Gly Ile Asp Val Lys Glu Ala Asp Pro Thr Gly His Ser Tyr Val Leu Val (SEQ 2)

Alignment of peptides with MAGE-Al/SEQ ID NO:2 (residues 145-172 shown)

|            |         |      | Glu /  |
|------------|---------|------|--------|
|            |         |      | Αla    |
| <b>ABD</b> | 100     | 2    | Asp    |
| Pro        | , 4     | 5    | Pro    |
| Thr        | TOT     | 1    | Thr    |
|            |         |      | 4T9    |
|            | His     |      |        |
| 20         | Ser     | (    | N<br>D |
|            | Tyr     |      |        |
| 17         | Val     | V CL | 5      |
| 1          | Val Leu | Fett | 1      |
|            | Val     | ۲a۷  |        |
|            | (SEO 6) | (SEC |        |
| ,          | 5       | 5    |        |
|            |         |      |        |

| E V         | 1      |      |         |
|-------------|--------|------|---------|
| G T G       | 3 5    | 3    |         |
| ETA.        | 1 2    | ¥    |         |
| Ala Asp Pro | day    |      | ABD     |
| Pro         | Pro    |      | Pro     |
| Thr         | Thr    |      | 7       |
| Gly         | Gly    | X T. | 3       |
| His         | His    | P L  | ۲.<br>۱ |
| Ser         | Ser    | Der  | 2       |
| TYr         | Tyr    | TYT  | 1       |
|             |        | Val  | 1       |
|             |        | Leu  | 1       |
|             |        |      | 1       |
|             | (SE)   | (SEO | 1       |
| 0 0         | ָ<br>פ | 2    |         |

| ¢        | , C                    | Ė                                       | 2                                       | •     |
|----------|------------------------|---|---|-------|
| Ala      | Ala                    | ALa                                     | Ala                                     | N T & |
| Asp      | Asp                    | Asp                                     | Ala Asp Pro                             | Asp   |
| Pro      | Pro                    | Pro                                     | Pro                                     | Pro   |
| Thr      | Thr                    | Thr                                     | Thr                                     | Thr   |
| Gly      | Gly                    | Gly                                     | СŢĀ                                     | Gly   |
| His Ser  | Hig                    | His                                     | His                                     | His   |
| Ser      | Ser                    | Ser                                     | Ser                                     | Ser   |
| TYT      |                        |   |   |       |
|          |                        |   |   |       |
|          |                        |   |   |       |
|          |                        |   |   |       |
| (SEQ 53) | ה לם<br>מו לם<br>מו לם | 2 | 2 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 | (SEC) |
| 53)      | 1 5                    | 14)                                     | 1 5                                     | 1 1   |
|          |                        |   |   |       |

Xaa G1

## Alignment of peptides with MAGE-A3/SEQ ID NO55 (residues 163-185 shown)

Gly Ile Glu Leu Met Glu Val Asp Pro Ile Gly His Leu Tyr Ile Phe Ala Thr Cys Leu Gly Leu Ser (SEQ ID NO:55) 180

Glu Val Asp Pro Ile Gly His Leu Tyr Met Glu Val Asp Pro Ile Gly His Leu Tyr Ile Phe Ala Thr Cys Leu Asp Pro Ile Gly His Leu Tyr Ile Phe

Met Glu Val Asp Pro Ile Gly His Leu Tyr (SEQ ID NO:56) (SEQ ID NO:57) (SEQ ID NO:58)

SEQ ID NO:56 aligns with residues 168-176 of SEQ ID NO:55 SEQ ID NO:57 aligns with residues 167-182 of SEQ ID NO:55 SEQ ID NO:58 aligns with residues 170-178 of SEQ ID NO:55 SEQ ID NO:59 aligns with residues 167-176 of SEQ ID NO:55